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Hemoglobin in the Symbiont-Harboring Gill of the Marine Gastropod *Alviniconcha hessleri*

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Hydrogen sulfide of geochemical origin, mixing at oceanic hydrothermal vents with oxygen from oceanic seawater, supports dense populations of chemoautrophic, sulfur-oxidizing bacteria. Those animals, the vestimentiferan worm Riftia pachyptila, *certain bivalve molluscs, and the recently discovered Pacific gastropod* Alviniconcha hessleri, *that interiorize the bacteria as intracellular symbionts dominate the vent fauna (1, 2). The immense size of these animals, the large standing crop represented in their dense communities, and the rapid growth of individuals all attest to the effective use of an abundant food base. Dense concentrations of the mesogastropod* Alviniconcha hessleri *(2, 3) were recently discovered at deep-sea hydrothermal vents at the spreading center in the Mariana Back-Arc Basin of the Western Pacific. These animals house chemoautrophic, sulfide-oxidizing bacteria within specialized cells (bacteriocytes) of their modified gills (2). They are the only reported example of a symbiotic association between a gastropod mollusc and intracellular chemoautotrophic bacteria. We now show that the modified gill of* Alviniconcha *contains hemoglobin at a concentration of about 65 μmol/kg wet weight gill. This value falls within the range, 20–250 μmol hemoglobin per kilogram, encountered in the modified symbiont-harboring gills of many of the sulfide-dependent clams examined but is short of the very high concentrations, 550 and 1200 μmol/kg, found in* Myrtea spinifera *and* Lucina pectinata *respectively (4). Accordingly, bacteriocyte hemoglobin is a feature common to both gastropod and bivalve symbioses.*

Symbioses with intracellular carbon-fixing bacteria, believed to be dependent on bacteriocyte hemoglobin, have heretofore been described only in clams of the families Solemyidae, Lucinidae, and Vesicomyidae and in a few mussels, Mytilidae, restricted to the genus *Bathymodiolus* (4). The molluscan symbionts fix carbon from oceanic carbon dioxide into hexoses and supply almost all of the carbon nutrition of the host (5). Ribulose bisphosphate carboxylase/oxygenase (RuBisCO), the enzyme responsible for carbon dioxide fixation, has been cloned from the *Alviniconcha* symbiont and expressed in *Escherichia coli* (6). The relatively low specificity of the purified enzyme for carbon dioxide indicates that the intracellular environment of the endosymbionts may be microaerophilic for RuBisCO to maintain net carboxylation (7).

Hemoglobins, probably located in the host cytoplasm and excluded from the peribacterial space and probably coded by host genes, are a near-constant feature of symbioses between bivalve molluscs and intracellular chemoautotrophic bacteria (4, 8); such hemoglobins are also a constant feature of symbioses between plants and intracellular prokaryotic nitrogen-fixing symbionts (9). Clam gill hemoglobins have been investigated intensively (10–13), and the three-dimensional structure of one is known from x-ray diffraction analysis (14). The probable role of the hemoglobin is to bring oxygen and hydrogen sulfide to the symbiont (8, 15). In the giant tube worm *Riftia*, this function is served by blood or coelomic hemoglobin, which bathe the symbiont-harboring cells and transport oxygen at the heme and sulfide at a site remote from the heme (16). In the bacteria-housing clam gill, these functions are probably served by two separate hemoglobins of the bacteriocyte cytoplasm (17). Cytoplasmic hemoglobins are believed to supplement the diffusion of free oxygen by adding to it a contribution from oxygen combined with the protein. In those few hemoglobin-containing tissues that have been studied intensively, the hemoglobin is maintained partially desaturated with oxygen, the larger part of the oxygen flow to the intracellular organelle is carried in combination with the protein, and

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the oxygen pressure is held low (18). This is in accord with the suggestion that low oxygen pressure is probably required to allow RuBisCO to maintain net carboxylation in the *Alviniconcha* gill (7). The concentration of hydrogen sulfide likewise is probably low, perhaps in the nanomolar range (8), and the concentration of free hydrogen sulfide may not be sufficient to support the flux of hydrogen sulfide to the symbiont.

A specimen of the Western Pacific hydrothermal vent gastropod *Alviniconcha hessleri* was collected by the submersible *Alvin* at the "Snail Pit" site (18° 10.95′ N, 144° 43.20′ E, about 3650 m depth) on Dive number 1837, 23 April 1987. The gill, 0.58 g wet weight, was stored frozen in liquid nitrogen and a clear soluble extract prepared in 1.5 ml of buffer (19). Optical direct spectra of this extract display a prominent narrow feature at 551 nm. This unchanging feature ascribed to reduced soluble symbiont bacterial cytochrome c_{552} (20), vanishes in the difference spectra. Optical difference spectra (Figs. 1 and 2)unequivocally establish the presence of hemoglobin in the extract. To confirm the identity of the spectral entities, optical difference spectra of the extract of *Alviniconcha* gill are compared to those of purified *Lucina* Hb II, isolated from the modified symbiont-harboring gill of the clam *Lucina pectinata* (10). The concentration of *Alviniconcha* hemoglobin in the solution, about 19 μM (heme), was estimated from the spectrum presented in Fig. 2A by using molar extinction coefficients appropriate for the difference: carbon monoxide *Lucina* Hb II minus ferrous *Lucina* Hb II. Optical spectra in the visible and soret regions are expected to differ only slightly (about ±10%) among similar hemoglobins. The concentration in the tissue was estimated (19) to be about 65 μmol *Alviniconcha* hemoglobin per kilogram wet weight gill.

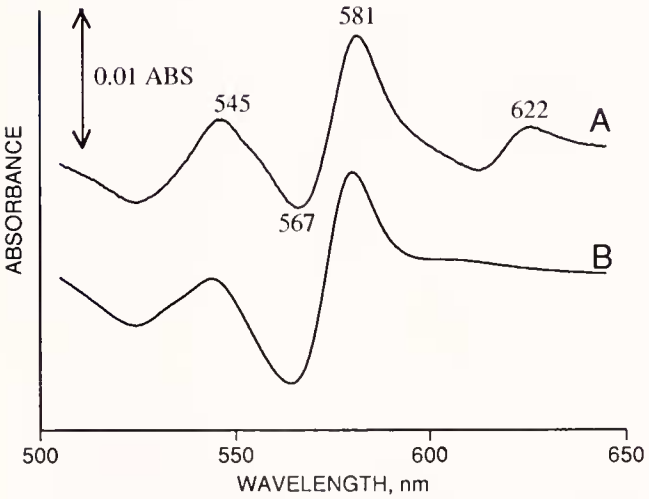

Figure 1. Optical difference spectra of the extract of *Alviniconcha* gill. Features ascribable to oxyhemoglobin were inconspicuous in the direct spectrum of the extract, suggesting that some component of the solution had consumed the dissolved oxygen. Accordingly the solution was equilibrated with oxygen gas. The difference spectrum of the oxygenated solution minus that of the initial (deoxygenated) solution (spectrum not shown) exhibits conspicuous features at 541 and 579 nm, diagnostic of oxygenated hemoglobin. A small feature near 622 nm suggested the presence of ferric hemoglobin. This feature increased in magnitude with time. The difference spectrum in the Soret region (spectrum not shown) of a portion of the sample that had been stored for 60 min at ice temperature minus that of a portion of the solution to which sodium dithionite (a reagent that removes oxygen and reduces most hemeproteins) had been added resembled the difference spectrum of ferric minus deoxy *Lucina* Hb II, and exhibited a maximum at 409 nm and a conspicuous minimum at 434 nm, diagnostic of hemoglobin. This confirms the presence of a hemoglobin in the solution. The oxygenated solution was then equilibrated with carbon monoxide. (A) Difference spectrum: Carbon-monoxide-equilibrated gill extract minus that of the oxygenated extract. Features at 545, 567, and 581 nm are diagnostic of hemoglobin. Conversion of oxy- to carbon monoxy hemoglobin shows that oxygen binding by the hemoglobin is reversible. (B) Difference spectrum: Carbon monoxide minus oxy *Lucina* Hb II.

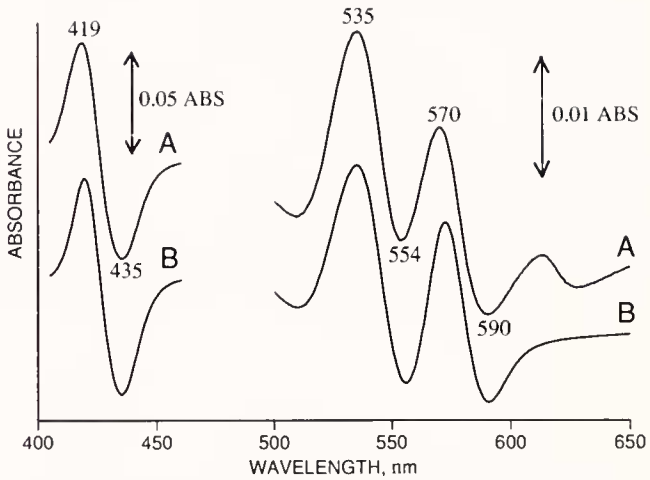

Figure 2. By adding sodium dithionite, all of the hemoglobin present in the extract of *Alviniconcha* gill is converted into single chemical species, permitting quantitative estimation of concentration. Absorbance in the visible region is amplified sixfold relative to the Soret region. (A) Difference spectrum: Carbon-monoxide-equilibrated gill extract containing sodium dithionite minus the same extract containing sodium dithionite alone. Well-defined features of 419, 435, 535, 554, 570 and 590 nm are diagnostic of hemoglobin. (B) Difference spectrum: Carbon monoxide *Lucina* Hb II minus deoxy (ferrous) *Lucina* Hb II.

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